UNIVERSITY OF GONDAR

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES

DEPARTEMENT OF IMMUNOLOGY AND MOLECULAR BIOLOGY

LEVEL OF ZINC, ALBUMIN AND IMMUNOLOGICAL MARKERS ON NEWLY DIAGNOSED TUBERCULOSIS PATIENTS AT THE UNIVERSITY OF GONDAR HOSPITAL, NORTH WEST ETHIOPIA.

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THESIS SUBMITTED TO THE DEPARTEMENT OF IMMUNOLOGY AND MOLECULAR BIOLOGY, SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES, UNIVERSITY OF GONDAR FOR THE PARIAL FULFILLMENT OF THE REQUIREMENT FOR DEGREE OF MASTER OF SCIENCE IN IMMUNOLOGY.

June, 2014

Gondar, Ethiopia

CERTIFICATION

This is to certify that the thesis entitled **'Level of Zinc, Albumin and Immunological Markers on Newly Diagnosed Tuberculosis Patients at the University of Gondar Hospital, North west Ethiopia' submitted by MULUALEM LEMMA** for the award of MSc. degree in Immunology was carried out under our supervision and the thesis has not been previously submitted in part or full for any degree or diploma of this or any other University

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Examiner _____

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June, 2014

Gondar Ethiopia

DECLARATION

The research work in this thesis entitled "**Level of Zinc and Immunological Markers on Newly Diagnosed Tuberculosis Patients at the University of Gondar Hospital, North west Ethiopia**." was carried out by me under the supervision of Dr Ebba Abate (principal advisor), and Mr. Meseret Workineh (Co-advisor) in the College of Medicine and Health Sciences, School of Biomedical and Laboratory Sciences, Department of Immunology and Molecular Biology University of Gondar, for the award of MSc degree in Immunology. I declare that this work is original and has not been submitted to any other University or Institution.

Mr. MULUALEML LEMMA _____

ACKNOWLEDGMENTS

My gratitude and respect goes to my advisors Dr. Ebba Abate and Mr. Meseret Workineh from the Department of Immunology and Molecular Biology for their invaluable comments and great contribution in all aspects throughout the development of this work.

I am also thankful for the Department of Immunology and Molecular Biology, School of Biomedical and Laboratory Sciences, for all types of supports given during the study programme.

I would also like to extend my acknowledgement to all staffs working at the TB clinic, and, Medical Laboratory Diagnostic facilities for all type of support they have given me during sample collection and laboratory analysis. In addition, I am indebted to the staffs at the Department of Analytical Chemistry, Faculty of Basic Sciences for allowing me to use their facility while measuring zinc level.

Last but not least, I would like to extend my acknowledgement to all the study participants without their involvement this study would have not been possible.

ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
Alb	Albumin
BCG	Bromcresol Green
BD	Becton Dickinson
BMI	Body Max Index
CD	Cluster of Differentiation
DC-SIGN	Dendrtic Cell Surface Intracellular adhesion molecules 3-Grabbing Non-Specific
DNA	Deoxribo nucleic acid
ELISA	Enzyme- Linked Immune sorbent Assay
EPTB	Extra pulmonary tuberculosis
FACS	Fluorescence Activated cell sorting
HIV	Human immunodeficiency virus
IL	Interleukin
IFN- γ	Interferon-gamma
LAM	Lipoarabinomannan
MHC	Major Histocompatibility complex
NOS2	Nitric oxide synthase
PTB	Pulmonary tuberculosis
RNA	Ribo nucleic acid
RNI	Reactive nitrogen intermediates
SPSS	Statistical package for Social Science
TB	Tuberculosis
TH	T helper- lymphocyte
TNF	Tumor necrosis factor
Zn	Zinc

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ABSTRACT

Background: Tuberculosis (TB) is still remains one of the main health problems globally. Based on the 2013 WHO report there are 8.6 million new TB cases per year. In Ethiopia, TB is responsible for major hospital admission and death like other TB endemic areas. Micro nutrients and immune status are the main determinate factors of the incidence and the severity of the disease in TB patients.

Objective: The main objective of this study was to determine serum zinc level and some immunological variables such as Albumin level, CD4, CD8 monocyte and neutrophil in newly diagnosed TB patients and healthy control group in Ethiopia.

Methods: A comparative cross- section study design was conducted from February to May 2014. Blood samples were taken from TB patients and controls for CD4, CD8, monocyte and neutrophil determination. Zinc and albumin were determined in serum. Stool sample was collected and examined for intestinal parasites microscopically using Formal ether concentration technique.

Results: A total of 50 TB patients and 50 controls were included in the study. Among TB patients 12(24%) were females and 38(76%) were males. The mean age of the study participants was 29.96±9.27 with a range of 18-57years. The mean levels of serum zinc TB Vs controls, ($p=0.026$), albumin of TB Vs control, ($p<0.001$) and CD4 of TB Vs control, ($p=0.007$) of TB patients were significantly lower than the controls. The mean Neutrophil TB Vs control, ($p= 0.021$) and monocyte levels TB Vs control, ($p= 0.019$) were higher in TB patients compared to controls. Age, gender, and TB types didn't show statistical association with serum zinc, albumin, CD4, neutrophil and monocyte levels. But, CD4 level was significantly associated with HIV infection. Increased levels of monocyte and CD8 level were associated with *Hook worm* and *schistosoma mansoni parasites*, respectively.

Conclusion: The low level of zinc and other immunological parameters in TB patients signify their role and importance to combat TB infection. An interventional study with appropriate sample size and design is warranted to clearly show the role of micronutrient during TB infection**.**

Key words; Albumin, CD4, CD8, Monocyte, Neutrophil, Tuberculosis and Zinc

1. INTRODUCTION

1.1. Global epidemiology of Tuberculosis

Tuberculosis (TB) is one of the major global health problems and in 2013 there were 8.6 million new TB cases and 1.3 million TB deaths (1). Of the reported deaths, 430,000 were HIV positive TB patients The 2013 World Health Organization (WHO) TB reports for different regions of the world shows that the south East Asian and African region show the highest incidence. In this report the African region of smear positive new TB cases was 600,355(47%), smear negative new TB case 345.947(27%) and extra pulmonary new cases 234,539(18%) (2).

1.2. Tuberculosis in Ethiopia

Ethiopia is one of the 22 high burden countries. Based on the national TB prevalence survey, smear positive TB among adults and all age group was found to be 108 and 63 per 100,000 populations, respectively. The prevalence of bacteriologically confirmed TB was found to be 156/100,000 populations and by extrapolations, the prevalence of all forms of TB in Ethiopia is estimated to be 240/100,000 populations (3). In Ethiopia the incidence of TB cases as WHO reported by 2013, shows that smear positive 47236(33%), smear negative 47.340(33%) and extra pulmonary 46,854(33%) (1).The mortality and the incidence were 16 and 240 per 100 thousands of population, respectively. The HIV prevalence in incident of TB cases was 18 percent (4).

1.3. Bacteriology of Tuberculosis

Mycobactria are slender, curved rods that are acid fast and resistant to acid, alkalis and dehydration. The cell well contains complex waxes and glycolipids. Multiplication on enriched media is very slow; it multiplies within the time of 18 to 24 hours. On the basis of growth rate, catalase and niacin production and pigmentation in light and dark, mycobactrerial are classified in to members of mycobacterial tuberculosis complex (*M. tuberculosis* , *M bovis*, *M africanum* and *M microtii* (5). Human TB is mostly caused by *Mycobacterium tuberculosis* but *M africanum* and *M bovies* can be the cause too (6).

1.4. Host susceptibility of Tuberculosis

Environmental factor, host genetic factors and factors within the pathogen are important in determining the progression of the disease (7). Large number of host genes are involved in susceptibility and resistance of mycobacterium and other infection (8). Susceptibility to TB in humans appears to be highly phylogenic with many loci implicated. Hetrogenecity of genetic and allelic association is frequently observed when comparing results between populations and has many causes including epistasis, where one gene interferes with or prevents expression of the other located at different locus (9). This genetic susceptibility to mycobacterial infection seen in tuberculosis can be explained by the host genes identified (10).

The environmental factors associated with TB are poverty, overcrowding, alcoholism, stress, drug addiction and mal nutrition. The disease is spread easily in overcrowding and badly ventilated places among peoples who are under nourished (11). Co-infection with other pathogens has also a great role in shifting and modulating the immune system. The clinical response of TB depends on an effective Th1 immune response. Different studies have shown that helminths co infected TB patients associated with increased interlukine-10 (IL-10), regulatory T- cells (12). This indicates that co-infection with intestinal helminth could down regulate the Th1 immune response required to combat TB infection.

1.5. Immune response to Tuberculosis

The TB associated glycolipid, lipoarabinomannan (LAM) and mycolic acid are responsible for many immunological features. The bacilli is recognized by immune cells through cell receptors like macrophage receptors, surfactant receptors, FC recptors, complement receptors and cluster of differentiation (CD1) molecules and DC-SIGN(13). Phagocytic cells play a key role in the initiation and direction of adaptive T-cell immunity by presentation of mycobacterial antigens and expression of costimulatory signals and cytokines. Alveolar resident macrophages are the primary cell type involved in the initial uptake of TB. Then, dendritic cells and monocyte derived macrophages also take part in the phagocytic process (14). Collaboration between macrophages and T-cells is essential for eradication of TB through antigen specific delayed type hypersensitivity response. Activated macrophage presents TB antigens to lymphocytes (T) cells in association with MHC class II molecules and macrophage derived cytokines play a critical role as co stimulatory molecules for antigen

specific T cells. Subsequently cytokines produced by activated T cell for further modulation of macrophages (15).

After phagocytosis of TB bacilli by macrophages and dendritic cells there is production of IL-12 it is used to leads the development of a Th1 response with production of interferon-gamma (IFN- γ) by T cell. Lymphocytes especially CD4+ T cells are the most important in the protective response against TB (16). A major effector mechanism responsible for the anti mycobacterial activity is IFN- γ, that induces the production of nitric oxide and related reactive nitrogen intermediates (RNI) by macrophages via the action of the inducible form of nitric oxide syntheses (NOS2) (17). Even if CD8 +T cells clearly cannot compensate for a lack of CD4+ T cells it also contribute to anti M. tuberculosis immunity , potentially by secreting IFN- γ to activate macrophages to control infection and by secreting products that can directly kill the M. tuberculosis bacilli (18).

After TB infection a dynamic process begins for the formation of granuloma and continuously evolves over time, the aggregation of white cells around the infected cells in the alveoli. Granuloma divided in to three distinct phase. The innate Granuloma, formed by loose aggregate composed primarily of recruited macrophage and neutrophil. The immune Granuloma, formed by following the emergency of antigen specific T cell and 'chronic Granuloma, results from distinct morphological change in Granuloma structure. The granuloma functions to limit the bacterial dissemination, but TB adapt to alter the immune response within the granuloma creating suppressive environment by IL-10 (19).

1.6. Micronutrients against Tuberculosis

Tuberculosis has greater impact on countries with low socio-economic levels especially affects labourers, and peoples living in overcrowded environment and poor housing (20). Micronutrients take the major role in the development and maintenance of immune response, and also affects both immediate and long term defence against infection and certain tumors (21). The occurrence of malnutrition in individuals with latent TB is one of the triggering factor for the development of active TB and it has been reported that malnutrition compromise cell mediated immune response (22). Several micronutrients such as zinc, vitamin A, riboflavin, folic acid, beta-carotene, vitamin B12, iron, Vitamin C, and selenium have immune modulating functions and thus influence the course and outcome of diseases and host susceptibility to infectious diseases (23).

Zinc is one of the micronutrients which have many functions. It is the fundamental factor determines the activity of many enzymes and forms the active enzymatic sites of many metallo-proteases. It is essential to the function of DNA polymerase, thymidine kinase and DNA dependent RNA polymerase. Zinc has also a great role in the activity of the transcriptional regulator family, nominated as zinc finger DNA binding proteins (24). Zinc is one of the basic requirements for the activity of some immune mediators. Thymulin is a nonapeptidic hormone secreted by thymic epithelial cells that promotes T lymphocyte maturation, cytotoxicity, and IL-2 production which requires the presence of zinc (25). Decreased naive T cells production from the thymus and decreased $CD4^+$, $CD45RA^+$ /$CD4^+$ $CD45RO^+$cells has been shown in zinc deficient individuals (26). Furthermore, zinc is useful for Innate immune defence mechanism and the highly proliferative organ of the immune system are influenced by zinc too (27).

The immune system is regulated mostly by zinc homeostasis in cells. Zinc deficiency results in reduced antibody production for neoantigens because of B cell is more affected than memory cells. Macrophage, granulocytes, and monocyte show reduced level of phagocytosis and intracellular killing activates. Diminished recruitment and total number of Neutophil reduction is shown in zinc deficiency (28).Th1 cytokines like IL-2, INF-γ and IL-12 which is generated by stimulated macrophage and monocyte are zinc dependant. But on the other hand Th2 cytokines are not affected by zinc deficiency with the exception of IL-10. IL-10 production is high during zinc deficiency in elderly individuals and simultaneously this increasing has an adverse effect on Th1 and macrophage function (29).

Zinc is very important for the production of cells and proteins in adequate level as stated earlier and the immune cells like CD4, CD8, monocyte and neutrophil are very crucial for our immune system to combat TB infection. However, there is lack of information regarding zinc level in association with some immunological markers and immune cells in Ethiopian TB patients. Therefore, this study was designed to provide some information and fill some of the gaps observed on the area.

2. LITREATURE REVIEW

The distinctive features of Zinc deficiency on immune system in human and in higher animal are thyimic atrophy, compromised cell function, lymphopenia and increased rate and duration of infection (30). These phenomena are supported by a number of research data in TB and other infectious and non-infectious diseases.

A study done in Pakistan among 117 patients of newly diagnosed pulmonary tuberculosis, 89 (76.01%) were males and the mean zinc level was 9.24±0.92mmol/l. Only 8(6.84%) have normal zinc level. Zinc level and sex of the study participant didn't show a significant association (p=0.220), but age was found significant (p=0.002) (31).

A study done in India on 20 TB and TB/HIV co infected patients. Their albumin and zinc level was determined for the two groups, the mean albumin level was 2.9±0.4g/dl and 3.6±0.7 g/dl, the mean zinc level was 53.9±8 and 65.53± 9.8µg/dl for TB/HIV co infected and TB patients respectively. Both zinc level and albumin level were significant ($P < 0.001$) (32).

A study was done in India on 50 TB patients and 30 controls .In this study the mean serum zinc level in TB patients was lower than the controls. A significant fall in zinc levels shown when compared with that of the control group ($p < 0.05$) (33).

Other study done in USA in 85 TB patients, 43.5% showed low CD4 cell counts and 56.4% patients had normal CD4 count. The CD8 cell count was lower in the patients with low CD4 cell counts than patients with normal CD4 cell counts ($P < 0.001$). Total lymphocyte count was also lower in patients with low CD4 cell counts than in patients with normal CD4 cell counts. The CD4: CD8 ratio was similar in both control and study subjects (34).

A study showed that Zinc-deficient patients had significantly depressed albumin concentrations of 3.23 ± 0.27 g/dl before zinc supplementation ($p < 0.001$). This mean increased to 3.95 ± 0.71 g/dl after zinc supplementation and was not statistically different from the control group (4.02 ± 0.34 g/dl) (35)

A case control study matched for age and sex was conducted in Indonesia on 41 active TB patients and 41 healthy control groups. The BMI in all patients was 20% lower than the controls ($p< 0.05$), serum albumin concentration was 10% lower in TB patients than in control($p<0.05$) and plasma zinc concentration in patients were significantly lower than those controls ($p < 0.05$)(36).

A study was done in India among TB and TB/HIV co infected patients with that of control groups. The CD4 count of the control (1773/ul) was much higher than TB patients (793/ul), TB/HIV co-infected patients (379/ul) and HIV patients (550/ul). Serum albumin and zinc level of the controls much higher than TB and TB/HIV co infected patients(37).

A study done in India on 39 TB patients, their CD4 counts is lower than when compared with normal blood donors ($p < 0.05$), but the CD8 value in TB patients similar with the controls (38)

A study done in Turkey among 75 active pulmonary TB, 25 inactive pulmonary and 20 healthy controls, the level of CD4 and CD8 counts in the active and inactive pulmonary TB patients were lower compared to the health controls, with p value of ($P < 0.01$) and (P 0.05),respectively (39).

A study done in India in TB patients with control groups, total lymphocyte and CD4 count were done for both groups. The values of CD4 were 971±376 and 2223±333 of for patients and controls, respectively ($p=0.002$).But CD8 value of both groups didn't show difference. (40).

A study done in Nigeria assessing the level of total protein and albumin in PTB patient they have found out that the levels of albumin and total protein in TB patients was lower than the controls. In the controls 8.38±1.1 and 4.99±0.5 and PTB patients 7.89±1.0 and 3.6±0.5 for total protein and albumin, respectively($p < 0.05$) (41).

A study done in South Africa on 43 newly diagnosed TB patients and the same amount of controls, the mean zinc level was 59.49 ± 10.96 and 77.03± 14.88were, respectively, ($p = 0.000$) (42).

A study done in Uganda among health controls and TB patients, absolute monocyte level of TB patients (2754±323) were higher than the controls (803±68) with ($p <0.001$), but CD4 and CD8 cell of the TB patients were lower than the controls but didn't show significant association (43).

A study done in South Africa among controls and TB patients, CD4 count were significantly depressed in TB patient relative to control subjects($P< 0·01$). Absolute number of monocyte and neutrophil count were elevated in the patients than the controls ($p<0.01$ and $p<0.05$).

CD8 counts were lower in TB patients than controls but did not show significant association (p= 0.13) (44).

A study done in Gondar Ethiopia among 112 newly diagnosed TB patients, 112 community controls and 71 house hold contacts, the median and inter quartile range of the CD4 in community control 750 and 627-923,(p=0.34). The house hold control CD4 median and inter quartile were and 714and 578-825, (p= 0.012). Which has high level compared to the TB patients' CD4 median and inter quartile range were, 513 and 390-682 respectively (45).

Another study in Gondar Ethiopia, determination of micro nutrients' among TB patients and control groups , the mean serum zinc level of TB patients was lower than the controls (p< 0.05)(46).

3. RATIONAL OF THE STUDY

There is lack of information regarding the level of zinc, Albumin level, Immunological markers and immune cells in TB patients in association with age, sex, body max index and other determinate factors in Ethiopia. This paper aims to address some of the gaps in this issue.

The findings of this work provide valuable information about the immunological markers, zinc level and albumin level among TB patients when compared to control group. This will be very vital information for the management of TB patients during treatment and follow up in addition other clinical findings.

The findings of this work gives data for individuals who want to do further study on TB patients to deal bout micro nutrients and immune status.

HYPOTHESIS

The working hypothesis of this study was the levels of Zinc, Albumin, CD4+Tcells,and CD8+T cells are lower in TB patients than healthy controls but immune cells (neutrophil and Monocyte) are higher than the controls.

4. OBJECTIVES

4.1. General Objectives

- Measuring the levels of micronutrients, immunological markers and immune cells in newly diagnosed TB patients and healthy controls.

4.2. Specific Objectives

- Measuring the serum zinc level in newly diagnosed TB patients and healthy controls.
- Measuring the albumin level in newly diagnosed TB patients and healthy controls.
- Measuring neutrophil and monocyte among the newly diagnosed TB patients and healthy controls.
- Measuring the immunological markers such as CD4 and CD8, among TB patients and healthy controls.

5. MATERIAL AND METHODS

5.1. Study area

The study was conducted at Gondar University Hospital (GUH). GUH is a tertiary-level teaching hospital located Northwest Ethiopia in Gondar town, which is 750 km far from Addis Ababa. Gondar town has an elevation of 2135 meter above sea level. Taking into account of the national population survey of 2007 the town currently would have more than 300,000 population (47).

5.2. Population

5.2.1. Source of population

Patients who visited the GUH for medical service were source of population.

5.2.2. Study population

All newly diagnosed TB patients who visited the TB treatment centre at GUH were the study population and equivalent numbers of apparently healthy control groups who didn't show any kind clinical manifestation started from before three months.

5.3. Study design and period

It is comparative cross- sectional study that was conducted from February 2014 to May 2014

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

- Newly diagnosed TB patients
- Age 18-65 years

5.4.2. Exclusion criteria

- Chronic renal disease
- Chronic liver disease
- Malignancy / cancer
- Myocardial infarction
- diabetes mellitus
- surgery during the last month
- Any acute injury (wound)
- Patients taking zinc as medication

5.5. Study variables

5.5.1. Dependant variables

- Zinc level
- Albumin level
- CD4+ cell level
- CD8+ cell level
- Monocyte cell level
- Neutophil cell level

5.5.2. Independent variables

- Age
- Sex
- Occupation
- Level of Education
- BMI
- Residence
- HIV
- Smear positive tuberculosis
- Smear negative tuberculosis
- Extra pulmonary TB
- Intestinal parasites

5.6. Sample size and Sampling techniques

It was a coverage survey in that all newly diagnosed TB patients who were diagnosed and visited the DOTS centre for TB treatment at the GUH during the study period and those who fulfilled the inclusion criteria were included in the study. Convenient sampling technique was applied.

5.7. Data collection and laboratory methods

Blood and stool samples were collected from the study participants. Ten ml of blood collected and of these 5 ml was used for the determination of CD4-T cells and CD8-Tcells levels using FACS Calibur machine and also used for haematological analysis. Five ml blood

was used for chemistry and serum zinc level measurement. The stool sample was examined microscopically using Formol ether concentration technique for intestinal parasites.

5.7.1. Laboratory methods

Flow cytometry

Surface marker staining of the lymphocytes (CD4 and CD8), were done by using by using specific monoclonal antibody, conjugated with fluorochrome day. The automated machine analyzed and counted the stained mono nuclear cells from the peripheral whole blood. The FACS calibur machine used BD cell Quest software for analysis (48).

Albumin determination

Serum albumin was determined after serum was separated in clinical chemistry laboratory. By using Bromcresol Green (BCG) method, Albumin in the serum sample mixed with the reagent that hold BCG chemical for the development of colored complex. The absorbance of the colored complex at 628nm directly proportional to the concentration of albumin in serum up to the linear limit of the method (49).The absorbance of the mixture was taken by MindrayBS200 full automated analyzer, and the result was obtained by multiplication of the absorbance value with the factor (50).

Zinc level determination

Serum sample for zinc determination was diluted in distilled water by using calibrated micro pipette in to1:10 diluted sample (51). In Atomic Absorption Spectroscopy (AAS) machine, the diluted sample converted in to atoms by atomization using acetylene gas. Zinc hollow cathode lamp was used as a light source .During atomization the valence electron of zinc atom, fluctuate from ground state to excited state. The ground state zinc atom absorb the light that come from the hollow cathode lamp, which had the same characteristic of wave length as emitted zinc electron returning from the exited state to the ground state. The intensity of the absorbed light is proportional to the concentration of zinc atom in the atomized solution. Five serially diluted know standard solution was done to develop factor, the level of zinc in the sample was obtained by multiplication of the developed factor from the standard with the absorbance of the sample and the dilution factor (52).

Total Monocyte and Neutrophil counts

The total monocyte were counted by Beckman Coulter ACT diff machine (53) and neutrophil count were done by using cell Dyn 1800 haematological analyzer from whole blood. Both The Cell-Dyn 1800 Haematology Analyzer and Beckman Coulter machine performs a Complete Blood Count (CBC) by using Electrical impedance principle (54).

Stool Examination

Stool examination was done by formal ether concentration technique. The patients were well oriented to bring appropriate sample volume and as much as they can to kept the sample from toilet contaminations. All the samples were preserved immediately by 10% formalin until examination. During concentration procedure the entire preserved sample we used as whole, microscopic reading was done by preparing two slides from single sample sediment by two laboratory technologists (55).

Body max index (BMI) of the study participants were calculated after taking weight and height. Classification was done based on WHO classification method(56). The age of the study participants were classified based on Sturges rule(57).

5.8. Quality controls

The entire instrument used (AAS, MindrayBS200, Beckman Coulter ACT diff, Cell- Dyn 1800 and Facs Calibur) were under the regular daily, weekly and monthly maintenance. Standard Operating Procedures (SOPS) were strictly followed during sample collection and handling, sample processing and analysis. Calibration was done when new batch of reagent applied before running quality control material. Quality control tests were run before running the test samples and latter the samples were run after verifying the quality control results.

The standardized questioners were prepared for collecting socio-demographic and clinical data, in English and Amharic language. All the collected data including laboratory finding was cheeked for completeness and entered to a computer on Statistical Package for Social sciences (SPSS) software (version 20)

5.9. Data analysis and interpretation

Descriptive statistics was used and the results were described as mean $\pm$ SD. Parametric tests such as student t-test and ANOVA were done for evenly distributed variables to assess the

the influence of age, sex, BMI and type of TB. For non-evenly distributed variables non-parametric tests such as, Mann-Whitney U test and Kruskall-walls tests were used. Variables with a p value < 0.05 was considered as statistically significant, all statistical analysis done by SPSS software (version 20)

5.10. Result dissemination

The final results have been disseminated to School of Biomedical and Health Science, University of Gondar, to University of Gondar hospital TB clinic. The findings will be written in manuscript form and will be submitted to a reputable journal for wider dissemination

5.11. Ethical consideration

Ethical approval was obtained from the ethics committee of the University of Gondar, Collage of Medicine and Health Sciences. Informed consent also obtained from all study participants.

6. RESULTS

Recruitment of study participants

A total of 100 study participants were included from February to May 2014. Of these, 71 were males and 29 were females. Seventy five participants were coming from urban area and the remaining 25 came from rural area.

Of the total 100 participants involved in this study, 50 were confirmed new TB patients cases and equal numbers of apparently healthy control individuals were recruited for comparison. The mean ± SD age of the total study participants included in this study was 29.9 ±9.3 years with range of 18-57 years. From the 50 TB patients included in this study 4 individuals were co-infected with HIV but they have not started anti retroviral therapy. Of the TB patients 66% (33/50) were pulmonary TB cases and the rest 36% (17/50) were patients with extra pulmonary TB. From the total Pulmonary TB patients 42% (14/33) were smear positive TB cases while 58% (19/33) were smear negative TB patients.

6.1. Socio demographic characteristic of study participants

From the total TB patients 12(24%) were females and 38(76%) were males. 24(48%) were farmers and 36 (72%) were not educated. (Table- 1)

Table 1. Socio demographic characteristic of the newly diagnostic TB patients and controls in Gondar, North west Ethiopia, 2014.

Variables	TB patients	Controls
Gender		
Females	12(24%)	17(38%)
Males	38(76%)	33(62%)
Residence		
Rural	25(50%)	0(0%)
Urban	25(50%)	50(100%)
Occupation		
Farmer	25(50%)	0(0%)
Daily labourer	9(18%)	2(4%)
Office work	2(4%)	34(68%)
House wife	7(14%)	0(0%)
Merchant	3(6%)	0(0%)
Student	4(8%)	14(28%)
Level of education		
Uneducated	36(72%)	0(0%)
High school	9(18%)	4(8%)
Diploma	4(8%)	11(22%)
First degree	1(2%)	29(58%
Above first degree	0(0%)	6(12%)
Religion		
Orthodox	45(90%)	42(84%)
Muslim	0(0%)	3(6%)
Protestant	5(10%)	5(10%)
Total no of participants	50	50

6.2. Prevalence of intestinal parasites

Form stool examination *Hook.worm* was the most prevalent (34%) parasites identified in TB patients.

Table 2. Prevalence of intestinal parasites in patients with active tuberculosis. Gondar, Northwest Ethiopia.2014

Intestinal parasite	Pulmonary TB			Extra pulmonary TB N (%)
	Smear positive N (%)	**Smear negative N (%)**	**Total N (%)**	
Hook worm				
Positive	7(14%)	5 (10%)	12(24%)	5(10%)
Negative	7(14)	14(28%)	21(42%)	12 (24%)
Schistsoma Mansoni				
Positive	2(4%)	4(8%)	6 (12%)	2 (4%
Negative	12(24%)	15(30%	27(54%)	15(30%)
Ascharise lubricoide				
Positive	2(4%)	6(12%)	8(16%)	1(2%)
negative	12(24%)	13(26%)	25(50%)	16(32%)
Stronglid.Stercolares				
Positive	1(2%)	1(2%)	2(4%)	0 (0%)
Negative	13(26%)	18(36%)	31(62%)	17 (34%)

6.3. Level of zinc, Albumin, immunological markers and immune cells

The mean serum zinc level was 12.52±5.98µmol/l and 17.29±9.58 µmol/l in TB patients and controls, respectively. The mean Zinc, Albumin and CD4+ cell levels were significantly lower in TB patients compared to controls, but the mean monocyte, CD8 and neutrophil were the reveres (Table 3).However CD8+ cell level didn't show any significant association.

Table 3. Level of zinc, Albumin, CD4 CD8, Monocyte, and Neutrophil in TB patients and healthy controls in Gondar, Northwest Ethiopia 2014**.**

	TB (mean± SD) n=50	**Controls (mean ±SD) n=50**	**P value**
Zinc	12.5±5.9	17.2±9.5	**0.026** (Mann-Whitney U test)
Albumin	2.7±1.3	5.3±0.6	**0.000** (t-test)
CD4	634±309	997±861	**0.007** (t-test)
CD8	703±632	618±255	0.659 (Mann-Whitney U test)
Monocyte	482±275	382±281	**0.019** (Mann-Whitney U test)
Neutrophil	**5416±3619**	**3964±1653**	**0.021 (Mann-Whitney U test)**

The mean of Albumin, CD4, CD8, Monocyte and Neutrophil didn't show any statistical significant with sex with the p-value of 0.654, 0.403, 0.660, 0.893 and 0.893, respectively. The forms of TB didn't show any statistical association with levels of zinc, albumin, CD8, monocyte and neutrophila (Table 4). Interestingly the CD4 level was significantly lower in pulmonary TB patients compared to extra pulmonary TB (p=0.001) (Table 4)

Table 4. Levels of zinc, Albumin, CD4, CD8, Monocyte and Neutrophil with forms of TB and type of pulmonary TB. Gondar, Northwest Ethiopia, 2014.

	Pulmonary TB			**Extra pulmonary TB (mean+SD)**	**p-value***	**p-value****
	Smear + (mean+SD)	Smear – (mean+SD)	Total (mean+SD)			
Zinc	10.9±6.0	12.9±6.2	12.2±6.0	12.95±6.15	0.263	0.631
Albumin	3.0±1.2	2.6±1.5	2.8±1.4	2.7±1.1	0.354	0.440
CD4	491±149	609±225	559±203	780±420	0.180	**0.000**
CD8	812±857	601±379	690±625	728±665	0.788	0.992
Monocyte	607±304	436±192	509±256	429±309	0.123	0.214
Neutrophil	4914±2367	5742±4874	5390±3976	5464 ±2912	0.760	0.674

*p-value between smear + and smear – TB

** P-value between Pulmonary TB (Total) and extra pulmonary TB

From the total TB patients 56% were having BMI < 18.5kg/m^2 but only 26% of the controls were having lower BMI (<18.5kg/m2). However there was no any association between BMI levels with Zinc, Albumin, CD4, CD8, Monocyte and neutrophil levels (Table 5). However, there was significant association between CD4 counts with BMI level, CD4 level of TB patients with BMI of < 18.5 kg/m^2 lower than TB patients with BMI of 18.5-24.9kg/m^2 (p = 0.047).

Table 5. Level of zinc Albumin CD4, CD8, monocyte and neutrophil with BMI in TB patients Gondar, Northwest Ethiopia, 2014

	BMI(<18.5kg/m^2)	**BMI(18.5-24.9kg/m^2)**	**P- value**
Zinc	13.78±6.04	11.27±5.86	0.241 (Mann-Whitney U test)
Albumin	2.61±1.33	2.99±1.29	0.367(t –test)
CD4+ Tcell	553. 39±237.91	737.72±361.78	**0.047**(t –test)
CD8+T cell	598.67±377.79	837.05 ±510.5	0.725(Mann-Whitney U test)
Monocyte	464.28±243.75	504 .54±315.43	0.767 (Mann-Whitney U test)
Neutrophil	5342.85±4457.82	5416.00±3619.24	0.207 (Mann-Whitney U test)

(Zinc= µmol/l, Albumin=mg/dl, CD4&CD8=cell/µl)

As shown in table 6, like that of sex there is no any significant association in the levels of Zinc, Albumin. CD4, CD8, monocyte and neutrophil with age group.

Table 6. Level of Zinc, albumin, CD4, CD8, Monocyte and Neutrophil with age group Of TB patient. Gondar Northwest Ethiopia, 2014.

Age group	18-22	23-27	28-32	33-37	38-42	43-47	48-52	53-57	P value
Zn	12.9±6.7	12.9±6.7	10.9±6.7	14.1±6.4	12.9±6.7	10.9±6.7	10.9±6.7	12.9±8.3	0.832
Alb	2.5±1.0	2.9±1.01	2.9±1.9	2.7±1.3	2.9±1.6	2.0±1.3	3.5±1.5	3.2±0.8	0.863
CD4	840±339	603±215	674±459	595±332	426±205	573±160	718±152	525±97	0.504
CD8	601.±213	1118±1121	964±944	659±587	498±337	578±148	466.±277	485±289	0.804
Mo	380.±204	671±275	671±407	400.±217	442.±237	425.±287	566±115	266±152	0.175
Neu	5310±3057	6671±2877	4500±1163	5155±912	6542±7949	6850±3321	3666±2948	2966±929	0.206

(Mo = monocyte, Neu = neutrophil, *Zinc= µmol/l, Albumin=mg/dl, CD4&CD8=cell/µl*)

6.4. Association of intestinal parasites and HIV with zinc, albumin, CD4, CD8, monocyte and neutrophil

Zinc and neutrophil didn't show any association with intestinal parasite and HIV, but as table 7 showed that their association between albumin and *Hook worm* (p-0.025), the mean albumin level is lower in *Hook Worm* positive than negative TB patients.CD4 and HIV ($p < 0.001$), CD8 and *schstosoma mansoni* (0.023) and monocyte with *Hook worm* (0.029), the mean monocyte level in *Hook Worm* positive were higher than in negative patients.

Table 7. Level of zinc, albumin, CD4+ cell, CD8+cell, monocyte and neutrophil with intestinal parasite and HIV Gondar Northwest Ethiopia.2014

	Hook worm			*Schist soma mansoni.*			*Ascaris.lumbricoied.*			*Strongyloide.stercolaries*			HIV		
	Positive n=17	Negative n=33	P value	Positive (n=8)	Negative (n=42)	p-value	Positive (n =9)	Negative (n=41)	p-value	Positive (n=2)	Negative (n=48)	p-value	Positive (n= 4)	Negative (n= 46)	p-value
Zn	10.6±5.7	13.6±6.0	0.555	12.9±8.3	12.4±5.9	0.894	9.0±4.8	13.5±6.0	0.236	12.9±8.3	12.5±5.9	0.894	14.9±6.7	12.2±5.9	0.780
Alb	2.7±1.7	2.8±1.0	**0.025**	3.2±1.6	2.7±1.2	0.145	3.0±1.4	2.7±1.3	0.949	3.0±0.3	2.8±1.3	0.138	2.4±0.7	2.8±1.3	0.546
CD4	722±357	588±276	0.139	569±276	834±409	0.069	689±236	622±324	0.420	386±42	645±311	0.197	214±158	671±293	**0.00**
CD8	653 ±657	739±638	0.661	1072±834	633±572	**0.023**	481±159	752±681	0.356	766±412	700±630	0.558	1166±751	663±614	0.153
Mo	582±273	430±266	**0.029**	550±358	469±259	0.427	422±238	495±283	0.426	450±71	483±280	0.940	425±170	486±283	0.850
Neu	5788±5416	5224±2375	0.525	5647±3825	4200±1991	0.223	7177±6724	5029±2479	0.318	5400±2121	5416±3682	0.686	5500±2290	5408±3729	0.593

(*Mo=monocyte,Neu=Neutrophila,Zinc=µmol/l,Albumin=mg/dl,CD4&CD8=cell/µl*)

7. DISCUSSION

This study assessed the level of zinc, Albumin, CD4, CD8, monocyte and neutophil in TB patients and the findings were compared with controls groups. The mean concentration of serum zinc level, Albumin, CD4 level were significantly lower in TB patients compared to controls. On the other hand, TB patients showed high Monocyte, Neutophil and CD8 cells levels than controls.

The low serum zinc concentration in TB patient in this study is in agreement with a study done in Pakistan (31), India (32) Indonesia (58). The probable cause for the lower serum zinc level in TB patients might be due to the reduction of zinc carrier proteins, α_2 - macroglobulin and or a rise in the production of metallothionein, a protein that transports zinc to the liver. The other probable reason could be nutritional factor as reflected in the BMI value in that large percentage of the TB patients were having BMI <18.5 compared to controls. Lower Zinc level may have an impact on macrophages functions such as phagocytosis, intracellular killing, cytokines production and, growth and function of T cells. Thus considering the fact that these immunological mechanisms are so vital to combat TB infection the lower level of zinc reported in this study raise a great concern.

In mice model it was demonstrated that zinc-deficient mice faced thymic atrophy, reductions in absolute number of splenocytes and depressed responses to both T-cell-dependent (TD) and T cell-independent (TI) antigens (59). In experimental human model zinc deficiency was correlated with lower IFN-γ production , where as the production of IL-4, IL-6, and IL-10 was not affected because of zinc deficiency (60). It is known that IFN-γ production is vital to combat TB infection. Even though this study didn't measure IFN-γ level the low zinc level observed in TB patients compared to healthy controls in the present study supports this fact. The association between gender with serum zinc level was assessed in patients with pulmonary tuberculosis. No significant association between gender and serum zinc level was observed in this study which is in agreement with a study done in Pakistan and India (31, 61). Furthermore other studies from Ethiopia (46) and India(62) was didn't show any association between serum zinc level with gender.

Albumin was significantly lower in newly diagnosed TB patients compared to the control group. The same finding was reported in Nigeria which demonstrated that three important nutritional indices (transferrin, total protein and albumin), were significantly lower in TB

patients compared to controls (41). The probable cause may be serum albumin concentration in malnourished patients is lower than that in well-nourished healthy controls. And also it was reported that TB patients with lower BMI exhibited lower albumin level (36).the other probable cause may be that the hepatic synthesis of acute phase proteins is induced by cytokines such as interleukin-6 and tumor necrosis factor which inhibit the production of serum albumin and cause dramatic shifts in the plasma concentration of certain essential micro nutrients and albumin (63). It is obvious that the production of certain cytokines such as IL-6 and TNF-α is increased during TB patients and this fits with the observed findings.

The level of CD4+ cells was also significantly lower in TB patients compared to control group. Similar findings were reported in different studies conducted in USA (34), India (37) and Ethiopia (45). Animal model study demonstrated that low CD4 count could be one of the main factor exacerbating development of active TB (64). It is well known that CD4+ lymphocytes are one of the main immune cells in cell mediated immune system that plays a great role to contain and kill TB bacilli (65). Its primary function is believed to be the production of IFN- γ and other cytokines to activate macrophages, which can then control or eliminate intracellular organisms such as TB baccilli (17). The main reason for having lower CD4 count in TB patients might be due to co-infection with HIV as the finding of this paper also demonstrated. It is clearly known that HIV co-infection rate is high in TB endemic areas and this will lead to have lower CD4 count as HIV mainly destroys these cells. The other probable reason might be due to low micro nutrients like zinc which is an essential micro nutrient for the development and function of important immune cells.

This study showed that the mean CD4 level of pulmonary patients was lower than extra pulmonary TB patients and showed significant association. Other findings were in the contrary (66, 67), the probable cause may be the prevalence of HIV in our TB patents much less than the others.

The mean CD8 cell level of TB patients was high when compared to controls but the difference was not statistically significant. This finding is in agreement with a study done by Afzal N et al (68) , Figen D, et al (39) and Gariby E, et al (69) and different from a study done by Handan H et al (68) and Thomas Cyt et al (70). Even if there was no association between CD8 level with TB patient, high level CD8 might be due to due to chronic parasite co infection, in addition to this our finding showed that significant association of *schistosoma mansoni* with CD8 level in TB patients.

In the present study, higher neutrophil count was observed and it was associated with TB. Similar findings were observed in a study done in Pakistan (71) and in Nigeria (72). However, this finding didn't ascertain whether increased neutrophil counts resulted from an increased in absolute numbers of neutrophil in the body or from trafficking of neutrophil in to the blood. The other probable cause of increased blood neutrophil level during TB infection might be due to noon specific inflammatory response of tissue damage during TB infection. Increasing blood neutrophil may not have a great role in combating TB infection because of a short life span and the bacilli grow in macrophages. As previously reported that increased neutrophil counts are common among patients with active TB disease in the context of poorly functioning acquiring immune response reported (73). The other probable reason might be the decreasment of production of INF-γ during TB infection. Because INF-γ inhibits the production of IL- 17 and other pro inflammatory cytokines, which are regulate neutrophil recruitment. Due to this neutrophila during TB infection may indicate loss or failed the Th1 immunity or INF-γ responsiveness. Study also showed that patient with grater sputum mycobacterium burden show high neutrophila (74).

In addition, monocyte in TB patients was higher compared to controls and the difference was statistically significant. This was similar with a study done in South Africa (44), and Saudi Arabia (75). Monocytes are the progenitor cell for macrophages and dendritic cells. Macrophage and dendritic cells is the main cell types involved during phagocytosis, production of nitric oxide species and IL-12 which is very important for activation of CD4 cell. The probable reason for blood level monocyte increase may be because during TB infection dramatically monocyte differentiation and acquisition in to effecter function are altered. The other possible reason might be the inflammatory cytokines like TNF-α production by the damaged tissue during TB infection cause monocytosis and monocytosis may be also due to the shifting of monocyte sub population from CD_{16}^{-} monocyte in to CD_{16}^{+}. CD_{16}^{+} type monocyte cells are poorly differentiated in to functional dendritic and macrophages, due to this blood monocyte level increased during TB infection.

8. CONCLUSION

Our study demonstrate that TB patients have low serum zinc level, albumin level and CD4+ T cell level when compared to the control groups on which all of them show significant association. On the other hand CD8+T cell monocyte neutrophil level in TB patients was higher than the control groups, but only CD8+ T cell didn't show any significant association. Interestingly, CD4+T cell levels significantly varied between pulmonary versus extra pulmonary TB in that significantly lower CD4+T cell level was observed in pulmonary TB patients. This statistical difference was not seen for other parameters such as zinc albumin, CD8, monocyte and neutrophil In addition, level of CD4+ T cell was associated with BMI, but no association was seen among age and gender with zinc, albumin, CD4+Tcell , CD8+T cell, monocyte and neutrophil. *Hook worm* was the prevailing intestinal parasites examined followed by *Schistosoma Mansoni*. From the dependant variables albumin and monocyte were show association with monocyte and CD8 show association with *Schistosoma Mansoni.*

9. RECOMMENDATION

- Zinc, albumin, and CD4 determination should be used as additional diagnostic tool for TB patients
- The low zinc level in TB patients compared to controls may urge the need for zinc supplementation.
- An interventional randomized study with zinc and nutritional supplementation is recommended to see the mere effect of supplementations to prevent TB disease and to evaluate its probable role for faster prognosis.
- The mechanism behind of significant difference in CD4 levels between PTB and EPTB should studies further.
- Moncytosis and Neutrophila in the blood of TB patients and their role for tissue reside intracellular pathogen should be Study.
- The high Hook worm rate in TB patients and its bad effect on vulnerable populations alerts the need to devise and promoting prevention tools such as improving personal hygiene and shoe wearing habit.

10. LIMITATIONS OF THE STUDY

The sample size and not being age sex matched in the study may have an influence on the power of the study.

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12. ANNEXS

Annex I; Laboratory procedure

Procedure I serum separation

1. Collect sample (preferably in glass tubes) and leave for an hour to allow it to clot.
2. Centrifuge the serum at 4000 -5000rpm for 5 minutes at room temperature.
3. Remove the serum from the clot by gently pipetting off into a clean tube using a glass Pasteur or micro pipette.
4. Label with appropriate material.
5. Store at -20°C.

Procedure II sample transportation

1. Prepare materials , ice pack , ice box and sample rack
2. Add the ice pack in every side of the ice box
3. Cover the sample in the sample rack by plastic bag
4. Add the covered sample in the prepared ice pox (sample container)
5. Add ice pack on the sample again and closed properly ice pox , and label with the kind of sample with biohazard mark.

Procedure.III. Serum zinc level determination by 210 flam atomic absorption spectroscopy

1. separate serum from whole blood by centrifuge at 3000 rpm for five minutes
2. Adjust the instrument by doing calibration by known control of sample and prepare calibration cure to make ready the machine for work
3. Dilute the serum in to 1:10 by distilled water to have enough amount of sample to the machine
4. Give the diluted serum sample to the machine properly without discontinuous.
5. Red the absorbance deference from the and obtain the net absorbance
6. Multiply the net absorbance by the factor to get result

Procedure, IV. Serum Albumin level determination procedure

1. Label test tubes Blank, Standard, Control and Patient
2. Pipette 1.5 ml of reagent into each tube
3. Transfer 0.01 ml (10 µl) of sample to respective tubes and mix and allow to stand at room temperature for five (5) minutes
4. Zero spectrophotometer with the blank at 630 nm. (Wavelength range: 580 – 630 nm).
5. Read and record absorbencies of all tubes
6. Abs. of unknown × Concentration of Standard = Albumin g/dl Abs. of standard

Procedure V. CD4, CD8, measurement procedure

1. Patient blood sample must be collected by k_3 EDTA about 3ml
2. Label the tab of the reagent with patients number
3. Vortex the pair upside down for 5 second and up right for 5 second
4. Open the reagents with the coring station
5. Mix the patients whole blood by inverting the tube five times
6. Pipette 50µl of whole blood in to each of the two reagents tubes , change tips between tubes
7. Cap the tubes and vortex up right for 5 seconds
8. Incubate the tubes for 60 to 120 minutes at room temperature (20^0 c to 25^0 c)
9. Uncap the tubes and pipette 50 µl of fixatives solution in to each of reagents tubes. change tips between tubes
10. Recap the reagent tube with new caps and vortex up right 5 second
11. Run the tubes on FACS count instrument within 48 hours

Annex II. Information sheet; English and Amharic version

Title of the Research Project

Immunological profile and Level of zinc on untreated tuberculosis patients in Gondar, North west Ethiopia

Name of principal investigator: Mulualem Lemma (BSC in medical laboratory)

Name of the Organization: University of Gondar College of Medicine and Health Science School of Biomedical and Laboratory Sciences Department of immunology and Molecular Biology.

Name of the Sponsor: University of Gondar

Introduction; the aim of this research is to measure zinc level and immunological profiles in newly diagnosed TB patients. This information sheet and consent form is prepared for explaining about the research to ask to participate in this research group.

Purpose of the project:. The purpose of this project is to measure zinc level and immunological profiles in the participate blood in newly diagnosed tuberculosis patients in Gondar, Ethiopia

Duration:

The duration of this study will be from February 2014 to April 2014

Procedure

1. The study participant will be a wared properly about the study

2. If the study participant is allow himself (herself) to be engaged in the study , he / she will sign the consent form
3. The participants will fill the questioner
4. Based on the questioner inclusion criteria, if the participants full fill all the inclusion criteria he will give 15 ml of blood and stool sample

Risk and /or Discomfort

By participating in this research project you will not face any health problem other than a slight discomfort especially during giving blood. Sample collection will take about 15 minutes.

Benefits

Your participation will help us to know whether zinc level and immunological profiles is low or normal or not in newly diagnosed tuberculosis patients in Gondar. Therefore, knowing this level will be good information about TB patients and the will be give a good source for other research

Incentives/Payments for Participating

You will not be provided any incentives or payment to take part in this project.

Confidentiality

The information collected from this research will be kept confidential and information about you that will be collected by this study will be stored in a file. In addition, it will not be revealed to anyone except the investigator.

Right to Refusal or Withdraw

You have the full right to refuse from participating in this research. You can choose not to response some or all the questions and this will not affect you from getting any kind of health service. You have also the full right to withdraw from this study at any time you wish.

If you need additional information about this project you can contact either the principal investigator or advisors by the following addresses.

MULUALEM LEMMA - Principal investigator

Cell phone no: +251-918 735 022 E-mail address; mulualeml@yahoo.com

DR. EBBA ABATE – main advisor

Cell phone no : +251 9 11464024 E-mail: ebbaabate@yahoo.com

MR. MESERET WORKINEH - advisor

Cell phone no: +251- 9 18295706 Email: mwmesi@gmail.com

Information sheet Amharic version

ለምርምሩ ተሳታፊዎች የሚሰጥ መረጃ

የምርምሩ ፕሮጀክት ርዕስ ፡ በጎንር ዩኒቨርሲቲ ሆስፒታል አዲስ ከተመረመሩ የቲቢ በሽተኞች ላይ የዚንክ እና የ ኢሚኖሎጂካል ፕሮፊይል ለመለካት የሚደረግ ጥናት፡፡

የተመራማሪዉ ስም ፡ ሙሉአለም ለማ (የሁለተኛ ድግሪ ተማሪ)

የመስሪያ ቤቱ ስም ፡ በጎንደር ዩኒቨርሲቲ፣ የሕክምናና ጤና ሳይንስ ኮሌጅ፣የባዮሜዲካልና ላቦራቶሪ ትምህርት ቤት፣ ኢሚኖሎጂ እና ሞሎኪውላር ባዮሎጂ ትምህርት ክፍል፡፡

መግቢያ

አዲስ ከተመረመሩ የቲቢ በሽተኞች ላይ የዚንክ እና የኢሚኖሎጂካል ፕሮፊይልን ለመለካት ጥናት ላይ ነኝ፡፡ እርስዎፈቃደኛ ከሆኑ በጥናቱ እንዲሳተፍ ተጋብዘዋል፡

ጥናትና ምርምሩ ዓላማ

የጥናትና ምርምሩ ዓላማ አዲስ ከተመረመሩ የቲቢ በሽተኞች ላይ የዚንክ እና የኢሚኖሎጂካል ፕሮፊይልን መለካት ነዉ፡፡

ጥናትና ምርምሩ የሚፈጀው ጊዜ፡ ጥናትና ምርምሩን ሰርቶ ለማጠናቀቅ አራት ወር ይፈጃል፡፡

ጥናትና ምርምሩ ሒደት

1. በመጀመሪያ ጥናት ዉስጥ የሚሳትፉ ሰወች ስለጥናቱ ማብራሪያ ያደረግላቸዋል
2. ከማብራሪዉ በኋላ ተሳታፊ ለመሆን ከተስማሙ የመስማሚያ ፎርሙ ላያ በፊርማቸዉ ያረጋግጣሉ
3. በወረቀት የተዘጋጀዉን መጠይቅ ይሞላሉ
4. በመጠይቁ መሰረት ሙሉ ለሙሉ የጥናቱ ተሳታፊ ከሆኑ የደም እና የአይነምድር ናሙና ይሰጣሉ

የሚያስከትለዉ ጉዳት

በጥናትና በምርመሩ በመሳተፍዎ ከመጠነኛ የእጅ ህመም ዉጭ ምንም አይነት የጤና ችግር አያስከትልም፡፡ ለምርምሩ ናሙና ለመሰብሰብ አስራአምስት ደቂቃ ብቻ ይወስዳል፡፡

ለተሳታፊዉ ወይም ለህብረተሰቡ የሚሰጠዉ ጥቅም

የጥናቱና ምርምሩ ዉጤት የዚንክ እና የኢሚኖሎጂካል ፕሮፊይል መጠን ዝቅተኛ ወይንም በቂ ወይንም ከፍተኛ መሆኑን ለማወችላል፡፡ ያይህንን መጠን ማወቅ ለትቢ ታካሚዎች እንደጥሩ መረጃ ሁኖ የጠቅማል፡፡ ለሊሎች ጥናቶች እንደ ጥሩ መረጃ በመሆን ይጠቅማል

የካሳ ክፍያ

ዕርስዎ በዚህ ጥናትና ምርምር በመሳተፍዎ ምንም አይነት የማካካሻ ክፍያ አይከፈለዎትም፡፡

ሚስጢራዊነት

ከዕርስዎ የተወሰደ ማንኛውም መረጃ ሚስጥራዊነቱ የተጠበቀ ነዉ። ከተመራማሪው ውጭ ማንም ሰው መረጃውን እንዲያውቀው አይደረግም።

እራስን ከጥናቱ የማግለል መብት

ዕርስዎ በዚህ ጥናትና ምርምር መካተት በዕርስዎ ሙሉ ፈቃድ ሲሆን በጥናቱ ያለመካተትም ሆነ ከተካተቱም በኃላ ያለምንም ቅድመ ሁኔታ ፈቃደኝነተዎን የማንሳት መብትዎ ሙሉ በሙሉ የተጠበቀ ነዉ።ያልፈለጉትን ጥያቄ ያለመመለስ መብት አለዎት።

ተጨማሪ መረጃ ከፈለጉ

ስለጥናትና ምርምሩ ማንኛዉን አይነት ጥያቄ ካለዎት በማንኛውም ሰዓት ተመራማሪዉንም ሆነ አማካሪዎችን በሚከተለዉ አድራሻ ማግኘት ይችላሉ።

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Annex IV. Consent form; English and Amharic version

I have been informed about a study that plans determine the level of zinc level, Albumin level , total protein level and immunological profiles among newly diagnosed pulmonary tuberculosis patients in Gondar, which will help in investigating the extent to which zinc lvel affect the host immune immunological profiles response to tuberculosis. For the study I have been requested to give a blood and stool sample. I am informed that experienced health professionals according to the established aseptic procedure would do the blood and stool collection. Based on this, I have agreed to participate in the study. The investigator also informed me that the laboratory findings would be kept confidential and I can withdraw my consent to participate in the study at any stage.

I have been given enough time to think over before I signed this informed assent. It is therefore, with full understanding of the situation that I gave my informed assent and will cooperate in the course of the study.

Name of study participant----------signature----------date----------

Name of investigator----------signature----------date----------

Amharic version of consent form

የስምምነት ቅፅ

ያህ የጥናት በአዲስ የቲቢ በሽተኞች ላያ የዚንክ እና የኢሚኖሎጂካል ፕሮፊይል መጠንን ለመለካትየሚደረገመሆኑንአዉቂ

ስለ ጥናቱ ዓላማ ምርምር እና ሒደት በሚገባ ተገልጾልኝ ምንም ምክንያት ሳያስፈልገኝ ከጥናቱ ለማቆረጥ እንደምችል ተረድቻለሁ፡፡ ይህ የስምምነት ቅፅ በአፍ መፍቻ ቋንቋዬ አንብቤ/ ተነቦልኝ በትክክል ተረድቼ በራሴ ፈቃደኝነት በጥናቱ እንድካፈል ተስማምቻለሁ፡፡ ለዚህም በፊርማዬ አረጋግጣለሁ፡፡

የጥናቱ ተሳታፊ ስም----------ፊርማ----------ቀን------/------/-----

የተመራማሪዉ ስም----------ፊርማ----------ቀን------/------/----

Annex V. Questionnaire; English and Amharic version

This questionnaire is prepared to collect socio demographic and clinical data from newly diagnosed tuberculosis patients for the study which aims to determine zinc level and immunological profile in newly diagnosed tuberculosis patients in Gondar. You are selected to participate in this study just by chance. It will take about 15 minutes to interview questionnaire.

Questionnaire code number _____

woreda_____

Town _____

Kebelle _____

Tell phone_____

Pare I: socio - demographic data status

101	Age	_____
102	Sex	A. Male B. Female
103	Residence	A. Urban B. Rural
104	Occupation	A. Student B. office work C. daily labourer D. farmer E. Hose wife
105	Religion	A. Orthodox B. Protestant C. Muslim D. Other specify_____
106	Level of education	A. Illiterate B. High school C. Diploma D. First degree E. Above first degree

Part II: Clinical data

s.no	Questions	Response
201	Do you have diseases other than tuberculosis?	1. Yes **2.** No
202	If your response for question number 12 is yes, what type of disease you have?	1. Malaria 2. Hepatitis 3. Kalazar(coutanousleishimania) 4. Cancer **5.** Others specify _____
203	If she is female are you pregnant?	1. Yes **2.** No
204	Q no 203 is no, are you taking contraceptive?	1. Yes **2.** No
205	Do you have surgery within the past one month?	1. Yes **2.** No
206	Do you have a blood loss or donate blood within the past three month?	1. Yes **2.** No
207	Is this tuberculosis for the first time?	1. Yes **2.** No
208	Do you have a history of diabetes mellitus?	1. Yes 2. No
209	Do you have known chronic organ problem?	1. Yes 2. No
2010	If Q2011 answer yes which organ?	1. Liver 2. Kidney 3. Bone marrow 4. Heart

Laboratory findings record sheet

1. Serum zinc level _____
2. Albumin level_____
3. CD4 count_____
4. CD8 count_____
5. Nutrophile_____
6. Monocyte_____

Amharic version of questionnaire

መጠይቅ:

ይህ መጠይቅ የተዘጋጀዉ በሰሜን ኢትዮጲያ፣ በጎንደር ከተማ፤ በጎንደር ዩኒቨርስቲ ሆስፒታል አዲስ የሳንባ ቲቢ በሽታ ከተገኘባቸው ታማሚዎች ዉስጥ የዚንክ እና የኢሚኖሎጂካል ፕሮፊይል መጠን እና ስለታማሚዉ ማንነት የሚመለከቱ ሁኔታዎችን መረጃ ለመሰብሰብ ነዉ።።

መጠይቅ

የመጠይቅ ቁጥር----------

ወረዳ----------

ክትማ----------

ቀበሊ----------

ስለተሳታፊው ማንነት መረጃን የሚመለከቱ መጠየቆች

ተ. ቁጥር	መጠይቅ	መልስ
101	እድሜ	
102	ጸታ	1. ወንድ 2. ሴት
103	የሚኖርበት አካባቢ	1. ከተማ 2. ገጠር
104	ስራ	1. ተማሪ 2. የቢሮ ሰራተኛ 3. የቀን ሰራተኛ 4. ገበሬ 5. የቤት እመቤት
105	ሐይማኖት	1. ኦርቶዶክስ 2. ፕሮቴስታንት 3. እስላም 4. ሌላ(ይገለጽ ----------)
106	ትምህርት ደረጃ	1. ያልተማረ 2. ሁለተኛ 3. ዲፕሎማ 4. የመጀመሪያ ደግሪ 5. ከድግሪ በላይ

የበሽታ ምልክትና ተዛማጅ ሁኔታዎች

ተ. ቁጥር	መጠይቅ	መልስ
201	ከቲቢ በሽታ ዉጪ ታመዉ ያዉቃሉ?	1. አዎ 2. አላውቅም
202	ለ201ኛዉ ጥያቄ መልስዎ አዎ ከሆነ ምን ዐይነት በሽታ ነበር ታመዉ የነበሩት	1. ወባ 2. የጉበት በሽታ 3. ካላዛር(ቁንጭር) 4. ካንሰር 5. ሌላ (ይገለጽ--------
203	ሲት ከሆነች . ነፊሰጡር ነዉተ	1. አዎ 2. አያደለሁም 3. አላውቅም
204	ተራቁጥር 203 ለ ከሆነ የእርግዝና መቆጣጠሪያ ያወስዳሉ?	3. አዎ 4. አልወስድም
205	በዚህ አንድ ወር ዉስጥ ቀዶ ህክምና ተደርጎልዎተ ያዉቃል	1. አዎ 2. አያውቅም
206	በዚህ 3 ወር ዉስጥ የደም መፈሰስ ወያንም ደም መለገስ አጋጥመዎታል	1. አዎ 2. አያዉቅም
207	ለመጀመሪያ ጊዚ ነዉ ትቢ የያዘዎት	1. አዎ 2. አደለም
208	የስኩአር በሽታ አለበዎት	1. አዎ 2. አላውቅም
209	የዉስጥ አካል ክፍል ችግር አለበዎተ	1. አዎ 2. አላውቅም
2010	የተቁጥር መልስ አዎ ከሆነ የትኛዉ ነዉ	1. የጉነበት 2. የእንኩለላሊት 3. የየአጥንት መቅን 4. የልብ

Laboratory findings record sheet

1. Serum zinc level _____
2. Albumin level_____
3. CD4 count_____
4. CD8 count_____
5. Nutrophil_____
6. Monocyte_____